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(54) Title: METHODS OF INHIBITING THE HGF–Met–uPA–PLASMIN NETWORK

(57) Abstract

The present invention provides a method of treating a subject exhibiting a HGF/SF–induced Met–activated metastatic phenotype comprising administering geldanamycin or a geldanamycin derivative to the subject, whereby the geldanamycin or the geldanamycin derivative inhibit Met, thereby treating the subject exhibiting the HGF/SF–induced Met–activated metastatic phenotype.

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METHODS OF INHIBITING THE HGF-Met-uPA-PLASMIN NETWORK

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention provides methods for inhibiting the HGF-Met-uPA-Plasmin network.

BACKGROUND ART

HGF/SF is an effector of cells expressing the Met tyrosine kinase receptor (*Gherardi et al.* 1993. Hepatocyte growth factor/scatter factor (HGF/SF), the *c-met* receptor and the behavior of epithelial cells." Symp. Soc. Exp. Biol. 47:163-181; *Matsumoto et al.* 1992. "Hepatocyte growth factor: molecular structure, roles in liver regeneration, and other biological functions." Crit. Rev. Oncog. 3:27-54 and *Rubin et al.* 1991. "Hepatocyte growth factor/scatter factor and its receptor, the *c-met* proto-oncogene product." Biochim. Biophys. Acta 1155: 357-371). It is produced by mesenchymal cells and acts predominantly on cells of epithelial origin in an endocrine and/or paracrine fashion (*Sonnenberg et al.* 1993. "Scatter factor/hepatocyte growth factor and its receptor the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development." J. Cell Biol. 123:223-235 and *Stoker et al.* 1987. "Scatter factor is a fibroblast-derived modulator of epithelial cell mobility." Nature 327: 239-242). As its name implies, HGF/SF promotes the growth and/or scattering of various cell types. HGF/SF has also been shown to mediate other biological activities, including the formation of tubules (*Montesano et al.* 1991. "Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor." Cell 67:901-908) and lumens (*Tsarfaty et al.* 1992. "The met proto-oncogene receptor and lumen formation." Science 257:1258-1261), the promotion of angiogenesis (*Bussolino et al.* 1992. "Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth." J. Cell. Biol. 119: 629-641), the inhibition of

cell growth (*Higashio et al.* 1993. "Tumor cytotoxic activity of HGF/SF." Exper. Suppl. 65:351-368) and the conversion from a mesenchymal to an epithelial phenotype (*Tsarfaty et al.* 1994. "Met mediated signaling in mesenchymal to epithelial cell conversion." Science 263:98-101). *In vivo*, this ligand-receptor pair is believed to play a role in neural induction (*Streit et al.* 1995. "A role for HGF/SF in neural induction and its expression in Hensen's node during gastrulation." Development 121:813-824), kidney development (*Santos et al.* 1994. "Involvement of hepatocyte growth factor in kidney development." Dev. Biol. 163:525-529), tissue regeneration (*Matsumoto et al.* 1993. "Roles of HGF as a pleiotropic factor in organ regeneration." Birkhauser-Verlag, Basel), and wound healing (*Nusrat et al.* 1994. "Hepatocyte growth factor/scatter factor effects on epithelia. Regulation of intercellular junctions in transformed and nontransformed cell lines, basolateral polarization of c-met receptor in transformed and natural intestinal epithelia, and induction of rapid wound repair in a transformed model epithelium." J. Clin. Invest. 93:2056-2065) and is required for normal embryological development. (*Uehara et al.* 1995. "Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor." Nature 373:702-705).

HGF/SF-Met signaling is also important in tumor development and progression. Met was originally isolated as the product of a human oncogene, *trp-met*, which encodes an altered Met protein possessing constitutive, ligand-independent tyrosine kinase activity and transforming ability. (*Cooper et al.* 1984. "Molecular cloning of a new transforming gene from a chemically transformed human cell line" Nature 311:29-33). The coexpression of unaltered Met and HGF/SF molecules in the same cell, which generates an autocrine stimulatory loop, induces an oncogenic transformation of those cells. (*Bellusci et al.* 1994. "Creation of a hepatocyte growth factor/scatter factor autocrine loop in carcinoma cells induces invasive properties associated with increased tumorigenicity." Oncogene 9:1091-1099).

In addition to transforming cells, deregulated Met signaling in cells increases their invasiveness *in vitro* (*Giordano et al.* 1993. "Transfer of mitogenic and invasive response to scatter factor/hepatocyte growth factor by transfection of human MET protooncogene." Proc. Natl. Acad. Sci. USA 90:649-653) and metastatic potential *in vivo* (*Rong et al.* 1994. "Invasiveness and metastasis of NIH/3T3 cells induced by Met-HGF/SF autocrine stimulation." Proc. Natl. Acad. Sci. USA 91:4731-4735). HGF/SF-Met signaling also induces the invasiveness and metastatic potential of other cell types (*Bellusci et al.* 1994). The detection of significant levels of HGF/SF in the pleural effusion fluid of patients whose cancer had metastasized to the pleura (*Kenworthy et al.* 1992. "The presence of scatter factor in patients with metastatic spread to the pleura." Br. J. Cancer 66:243-247) demonstrates the involvement of HGF/SF-Met signaling in promoting metastasis in humans.

For example, although HGF/SF is synthesized by mesenchymal cells and acts predominantly on Met-expressing epithelial cells, it has been demonstrated that human sarcoma cell lines often inappropriately express high levels of Met and respond mitogenically to HGF/SF (*Rong et al.* 1995. "Met proto-oncogene product is overexpressed in tumors of p53-deficient mice and tumors of Li-Fraumeni patients. Cancer Res. 55:1963-1970 and Rong et al. 1993. "Met expression and sarcoma tumorigenicity." Cancer Res. 53:5355-5360). It has also been shown that clinical sarcoma samples may overexpress the Met receptor (*Rong et al.* 1993 and *Rong et al.* 1995). Thus, this receptor-ligand pair is known to be involved in human oncogenesis and HGF/SF-Met signaling dramatically induces the *in vitro* invasiveness and *in vivo* metastatic potential of cells. In addition to its oncogenic potential, the HGF/SF-Met signaling results in elevated levels of cell-associated the urokinase type plasminogen activator (uPA) and enhanced plasmin-generating ability of human cells (SK-LMS-1). Therefore there is some association of the HGF/SF-Met signaling and the activation of cellular invasion-metastasis with the uPA-plasmin network.

Specifically, HFG/SF is a substrate for uPA, a serine protease of limited specificity (*Blasi* 1993. "Urokinase and urokinase receptor: a paracrine/autocrine system regulating cell migration and invasiveness." Bioessays 15:105-111 and *Ellis et al.* 1992. "The urokinase receptor: involvement in cell surface proteolysis and cancer invasion." Annu. N.Y. Acad. Sci. 667:13-31). Active uPA is found predominantly at the cell surface, where it is retained by a high-affinity receptor (uPAR). HGF/SF is secreted as an inactive monomer that is cleaved into its biologically active heterodimeric form by uPA (*Naldini et al.* 1992. "Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor." EMBO J. 11:4825-4833). In addition, uPA is involved in the degradation of the extracellular cellular membrane/basement membrane (ECM/BM) which is an important aspect of cellular invasion-metastasis, by virtue of its ability to activate plasminogen to the broad-specificity serine protease plasmin. Like uPA, active plasmin is predominantly a cell surface-associated protease, but its broader specificity enables it to play a more direct role in ECM/BM degradation than uPA. Since uPA plays a central role in catalyzing ECM/BM degradation, a strong association between uPA expression, and therefore the level of active plasmin, and the HGF/SF-induced invasive-metastatic phenotype therefore exists. Although the precise intermediate steps between HGF/SF-induced metastatic phenotype and the presence of active plasmin are not all known, the association between these two components allows one the unique ability to administer to a cell a compound that can inhibit an HGF/SF-induced metastatic phenotype and detect this inhibition effect by monitoring active plasmin. Where a compound interferes with an HGF/SF-induced metastatic phenotype of a cell, the level of active plasmin in that cell correspondingly decreases. The present invention provides for the first time such an assay, whereby a compound can be screened for its ability to inhibit an HGF/SF-induced metastasis by monitoring the level of active plasmin in a cell. This simplified assay represents a significant improvement over the prior art and greatly increases ones ability to screen vast numbers of compounds for that desired effect.

Prior conventional assays for screening inhibitors of HGF/SF use tyrosine kinase activity as a means for determining ligand-receptor activation *in vitro*. In these ligand-receptor interaction studies, the amount of ligand binding to the receptor is monitored and correlated to the effectiveness of a potential inhibitor. These methods are complex and cumbersome and generally require immunoprecipitation of the ligand-receptor complex for quantitation.

The method provided in the present invention provides a much simplified method of assaying or screening for inhibitors of the HGF/SF-induced tumorigenic phenotype by utilizing the activation of the urokinase plasminogen activator pathway. It is now possible to determine the uPA activity in viable cells without complicated multistep handling procedures and the assay uses a biological endpoint of HGF/SF-Met activation which has direct relevance to invasion/metastasis (i.e., production of proteolytic enzymes). One can therefore administer HGF/SF to cells in the presence or absence of a compound and readily determine the effectiveness of the compound to block or inhibit plasmin, and therefore HGF/SF-induced mitogenesis. This assay will therefore allow the rapid screening of compounds that potentially block or inhibit HGF/SF-induced mitogenesis. An additional benefit of this assay is that it not only directly assays the capacity of a compound to inhibit HGF/SF-induced mitogenesis, it is not necessary that the compound specifically block the immediate action of HGF/SF on the cell, such as interfere with the binding of HGF/SF to the Met receptor. Since all the intermediate steps between the action of HGF/SF and uPA are unknown, the mechanism of the blockage or inhibition can be anywhere within the potentially complex pathway between the initial effects of HGF/SF on a cell and the subsequent elevated level of active uPA in those stimulated cells (i.e., signaling components, level of uPA gene expression, activity, etc). Therefore the effectiveness of a potential inhibitor which may act at any point in that pathway can still be detected as a potential inhibitor of HGF/SF-induced mitogenesis with this assay.

The use of this assay has discovered eighteen compounds, specifically geldanamycin and derivatives of geldanamycin as being compounds that can inhibit an HGF/SF-SF-induced phenotype. These benzoquinoid compounds have previously been identified as having antibiotic properties and have been administered to patients, but prior to now, these compounds were not known to have any effect on an HGF/SF-induced metastatic phenotype. This clearly demonstrates the importance of the present invention for identifying additional compounds with such therapeutic potential and the use of geldanamycin and geldanamycin derivatives to treat a HGF/SF-induced metastatic phenotype.

SUMMARY OF THE INVENTION

The present invention provides the use of geldanamycin or geldanamycin derivatives for the manufacture of a medicament for inhibiting a hepatocyte growth factor/scatter factor (HGF/SF) induced phenotype in a cell.

Also provided are methods of treating a subject exhibiting a HGF/SF-induced Met-activated metastatic phenotype comprising administering geldanamycin or a geldanamycin derivative to the subject, whereby the geldanamycin or the geldanamycin derivative inhibit Met, thereby treating the subject exhibiting the HGF/SF-induced Met-activated metastatic phenotype.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Inhibition of HGF/SF-mediated MDCK cell scattering by NSC 122750 and 255109. MDCK cells were treated for 24 hours in media containing vehicle alone (A), vehicle plus 100U/ml HGF/SF (B), or 100U/ml HGF/SF in the presence of either 10 nM 122750 (C) or 255109 (D). Cells were fixed and stained prior to photography.

Figure 2. Inhibition of HGF/SF-induced SKLMS-1 chemotaxis (directed motility) by NSC 122750 and 255109. The ability of cells to migrate toward HGF/SF was assayed in a modified Boyden chamber in the presence or absence of either 100nM NSC 122750 or 255109.

Figure 3. The abilities of NSC 122750 and 255109 to revert the transformed phenotype of cells transformed by tpr-met, mutationally activated Trk-Met (3831/3997), mutationally activated Met (3831/3997), autocrine HGF/SF-Met overexpression and the V12-H-ras oncogene. Drugs (100 nM) or vehicle alone (controls) were added for 24 hours prior to photography.

Figure 4. Down-regulation of Met expression by NSC 122750 and 255109 in NIH3T3 cells expressing endogenous Met, tpr-met, mutationally activated Trk-Met (3831/3997), mutationally activated Met (383113997), and V12 H-Ras. Western immunoblot analysis was performed on cell lysates obtained following 24 hour treatment of cells with 100 nM of either NSC 122750 or 255109 or vehicle alone (controls).

Figure 5. Inhibition of HGF/SF-mediated urokinase (uPA) and uPA receptor (uPAR) induction in SKLMS-1 cells by NSC 122750 and 255109. Cells were treated for 24 hours with vehicle alone (controls, lane 1), 100 U/ml HGF/SF (lane 2) or HGF/SF plus either 50nM NSC 122750 (lane 3) or 255109 (lane 4) prior to western immunoblot analysis to determine expression of Met, uPAR and uPA.

Figure 6-23. Data from 18 geldanamycin analogues in the MDCK cell HGF/SF-Met-UPA screen. Both cytotoxicity (as % growth inhibition (%GI)) and inhibition of HGF/SF-induced uPA activity (as % Chromozyme inhibition (%CI)) are presented both graphically and in tabulated format for each of the analogues, together with their structure, NSC numbers, and other relevant information. (n>=3).

DETAILED DESCRIPTION OF THE INVENTION

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Example included therein.

Before the present compounds and methods are disclosed and described, it is to be understood that this invention is not limited to specific compounds and methods, as such may of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, a cell can mean a single cell of a population of cells.

The present invention provides a method of identifying a compound that inhibits a hepatocyte growth factor/scatter factor (HGF/SF) induced phenotype, comprising administering HGF/SF and the compound to a cell, administering a substrate for plasmin to the cell, wherein a cell containing active plasmin converts the substrate into a detectable product, detecting the presence of product converted from the substrate, whereby inhibition of conversion of the substrate into its product indicates an inhibition of the HGF/SF-induced phenotype, thereby identifying the compound as inhibiting a HGF/SF-induced phenotype.

Further provided by the invention is a method of identifying a compound that inhibits a hepatocyte growth factor/scatter factor (HGF/SF) induced phenotype, comprising administering a substrate for plasmin to a cell that expresses an HGF/SF-induced phenotype, wherein a cell containing active plasmin converts the substrate into a detectable product, detecting the presence of product converted from the

substrate, whereby inhibition of conversion of the substrate into its product indicates an inhibition of the HGF/SF-induced phenotype, thereby identifying the compound as inhibiting a HGF/SF-induced phenotype.

One skilled in the art will appreciate that identifying includes any means for identification or indicating a compound as having the capacity to inhibit an HGF/SF-induced phenotype. In this assay, this includes the capacity of the compound to decrease the level of active plasmin in a cell. Where a compound, when coadministered to a cell with HGF/SF, results in the decreased production of active plasmin, that compound is thereby identified as a compound that inhibits a HGF/SF-induced phenotype. Similarly, where a compound is administered to a cell that expressed a HGF/SF-induced phenotype and results in the decreased production of active plasmin, that compound is thereby identified as a compound that inhibits a HGF/SF-induced phenotype. Any means of monitoring the level of uPA activity, the level of inactive plasminogen, and/or the level of active plasmin can therefore be used to identify a compound, or composition, as an inhibitor of a HGF/SF-induced phenotype.

Similarly, one skilled in the art will appreciate that as used herein, the term inhibits means either an actual decrease in the expression of a HGF/SF-induced phenotype, or a decrease in the induction of that phenotype. Therefore, the HGF/SF-induced phenotype can actually continue to spread in a population of cells, but as long as that spread is at a level less than that before an inhibitory compound or composition is administered to the cell or expressed in the cell, that compound or composition inhibits the HGF/SF-induced phenotype.

The substrates for plasmin include any compound that can be altered by plasmin from one state into another state, or from a substrate to a product. For example, two specific substrates for plasmin include tosyl-glycyl-prolyl-lysine-4-nitranilide acetate and N-methylsulfonyl-D-phenylalanyl-glycyl-arginine-4-

nitranilide acetate. These substrates are cleaved by plasmin into a product that is readily detected.

For example, tosyl-glycyl-prolyl-lysine-4-nitranilide acetate, which is marketed under the trademark Chromozyme® PL (Boehringer Mannheim Corporation), is cleaved by plasmin into Tos-Gly-Pro-Lys-OH and p-nitraniline. One of the reaction products, p-nitraniline, can be detected spectrophotometrically at 405nm. One can therefore administer HGF/SF to a cell in the presence or absence of a compound or composition that is being screened for its capacity to block HGF/SF or inhibit a HGF/SF-induced phenotype, and readily determine the effectiveness of this compound by monitoring the conversion of tosyl-glycyl-prolyl-lysine-4-nitranilide acetate into Tos-Gly-Pro-Lys-OH and p-nitraniline.

In a specific example, cells can be treated with and without HGF/SF for 24 hours in the presence or absence of potential inhibitory compounds. Cells are then washed and incubated in media containing plasminogen together with a specific substrate for plasmin. Active uPA(or tPA) generated by HGF/SF stimulation cleaves plasminogen into the active protease, plasmin. Plasmin in turn cleaves the specific substrate tosyl-glycyl-prolyl-lysine-4-nitranilide acetate or N-methylsulfonyl-D-phenylalanyl-glycyl-arginine-4-nitranilide acetate, generating a chromogenic product which is easily read using an ELISA plate reader (absorbance at 405 nm). This assay can be performed in 96 well plates, allowing for screening of numerous compounds simultaneously and in multiples. The methods provided herein are therefore amenable to high throughput screening procedures.

In addition to spectrophotometric detection methods, the presence of plasmin can be detected by other methods described in the literature and well known in the art (*Jeffers et al* 1996. "Enhanced Tumorigenicity and Invasion-Metastasis by Heptocyte Growth Factor/Scatter Factor-Met Signalling in Human Cells Concomitant with Induction of the Urokinase Proteolysis Network" Mol. Cell Biol. 16: 1115-1125). Briefly, this method comprises incubating cells with or without

HGF/SF, supplementing the cells with ^{125}I-human plasminogen, harvesting the supernatants and the cell extracts, resolving the supernatant and cell extract proteins by SDS-PAGE and exposing the dried gels to X-ray film. This experiment would yield data indicating whether plasminogen has been cleaved into two fragments to produce active plasmin, and therefore demonstrate whether uPA has been activated by the administration of HGF/SF to the cells.

The methods provided by the present invention are therefore not limited to any specific detection protocol or detection technique, but can use any procedure which can detect the presence of active plasmin, inactive plasminogen, the activity of uPA, or any aspect or combination of steps in the plasmin-activation network or pathway.

Similarly, the cells used in the methods provided herein can be any cell which exhibits an increase in plasmin when administered HGF/SF or expresses endogenous HGF/SF. In one embodiment, the cells can comprise MDCK cells (dog canine kidney epithelial cells). Other cells displaying a response to HGF/SF include, but are not limited to HT-29, EMT-6, SKLMS-1, A431, and A549 cells.

In one embodiment of the present invention, the cell that is being monitored for the ability of a compound to inhibit an HGF/SF-induced phenotype can already express the phenotype. For example, the cell can express and produce HGF such that the cell exhibits the HGF phenotype and therefore administration of exogenous HGF is not necessary. These cells can therefore be used in a simplified version of the assay where one only needs to grow the cells, administer the compound or composition to be screened, and then monitor or detect active plasmin.

One skilled in the art will appreciate there are numerous methods of obtaining a cell already expressing a HGF/SF phenotype, such as using native cells expressing this phenotype or even transforming cells with a nucleic acid encoding HGF/SF such that expression of the nucleic acid results in the cell expressing a

HGF/SF phenotype. The specific identity of the cell is not limiting to the claimed invention and any cell or cell type that expresses a HGF/SF phenotype, whether typically or through manipulation of the cell, can be used in the methods claimed herein.

Also provided by the invention are compounds for administering to a subject to inhibit a HGF/SF-induced cellular phenotype, wherein the compounds comprise geldanamycin or a geldanamycin derivative other form of those compounds. Additionally, the present invention provides those compounds for use in the manufacturing of a medicament for inhibiting a hepatocyte growth factor/scatter factor (HGF/SF) induced cellular phenotype.

It is contemplated that the inhibitors of the present invention can be administered to cells or to a subject, most preferably, humans, to treat disease states which rely upon the activity of HGF/SF. The present HGF/SF inhibitor may be administered parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, topically, transdermally, or the like. The exact amount of such compounds required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disease or condition that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible or necessary to specify an exact amount. However, an appropriate amount may be determined by one of ordinary skill in the art using methods well known in the art (see, e.g., *Martin et al.*, 1989).

For topical administration, the compounds of the present invention can be in pharmaceutical compositions in the form of solid, semi-solid or liquid dosage forms, such as, for example powders, liquids, suspension, lotions, creams, gels or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions can typically include an effective amount of the selected compound in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal agents, pharmaceutical agents, carriers,

adjuvants, diluents, etc. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected compound without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. Since geldanamycin or geldanamycin derivatives have already been used medically to treat human subjects, one skilled in the art can readily determine the exact dosage of these compounds for administering to a subject.

Alternatively or additionally, parenteral administration, if used, is generally characterized by injection e.g., by intravenous injection including regional perfusion through a blood vessel supplying the tissues(s) or organ(s) having the target cell(s). Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Parenteral administration can also employ the use of a slow release or sustained release system, such that a constant level of dosage is maintained (*See*, for example, U.S. Patent No. 3,710,795). The compound can be injected directly to the site of cells or tissues expressing a HGF/SF phenotype, or they can be injected such that they diffuse or circulate to the site of the HGF/SF phenotypic cells.

Dosages will depend upon the mode of administration, the disease or condition to be treated, and the individual subject's condition. Dosages will also depend upon the composition being administered, *e.g.*, a protein, a nucleic acid, or another type of compound or composition. Such dosages are known in the art. Furthermore, the dosage can be adjusted according to the typical dosage for the specific disease or condition to be treated. Furthermore, culture cells of the target cell type can be used to optimize the dosage for the target cells *in vivo*, and transformation from varying dosages achieved in culture cells of the same type as the target cell type can be monitored. Often a single dose can be sufficient; however, the dose can be repeated if desirable. The dosage should not be so large as to cause

adverse side effects. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication.

For administration to a cell in a subject, the compound or composition, once in the subject, will of course adjust to the subject's body temperature. For *ex vivo* administration, the compound or composition can be administered by any standard methods that would maintain viability of the cells, such as by adding it to culture medium (appropriate for the target cells) and adding this medium directly to the cells. As is known in the art, any medium used in this method can be aqueous and non-toxic so as not to render the cells non-viable. In addition, it can contain standard nutrients for maintaining viability of cells, if desired. For *in vivo* administration, the complex can be added to, for example, a blood sample or a tissue sample from the patient, or to a pharmaceutically acceptable carrier, e.g., saline and buffered saline, and administered by any of several means known in the art. Other examples of administration include inhalation of an aerosol, subcutaneous or intramuscular injection, direct transfection of a nucleic acid sequence encoding the compound where the compound is a nucleic acid or a protein into, e.g., bone marrow cells prepared for transplantation and subsequent transplantation into the subject, and direct transfection into an organ that is subsequently transplanted into the subject. Further administration methods include oral administration, particularly when the composition is encapsulated, or rectal administration, particularly when the composition is in suppository form. A pharmaceutically acceptable carrier includes any material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected complex without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

Specifically, if a particular cell type *in vivo* is to be targeted, for example, by regional perfusion of an organ or tumor, cells from the target tissue can be biopsied

and optimal dosages for import of the complex into that tissue can be determined *in vitro*, as described herein and as known in the art, to optimize the *in vivo* dosage, including concentration and time length. Alternatively, culture cells of the same cell type can also be used to optimize the dosage for the target cells *in vivo*.

For either *ex vivo* or *in vivo* use, the compound or composition can be administered at any effective concentration. An effective concentration is that amount that results in reduction, inhibition, or prevention of transformed phenotype of the cells

The compound can be administered in a composition. For example, the composition can comprise other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. Furthermore, the composition can comprise, in addition to the compound, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

The present invention is more particularly described in the following example which is intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLE

Molecular Screen for Inhibitors of the Met-HGF/SF-uPA-Plasmin Network. To test the methods claimed herein, a plate format was designed such that 8 drugs may be simultaneously tested at three concentrations in triplicates in a 96 well plate format. The plate format also incorporates the necessary controls including +/- HGF in both the presence and absence of neutralizing anti-HGF antiserum. Replicate plates are run to determine cellular toxicity of compounds.

Cells growing in the wells of the plate were treated +/- HGF/SF for 24 hours in the presence or absence of potential inhibitory compounds. The cells were then washed and incubated in media containing plasminogen together with a specific substrate for plasmin. As discussed above, active uPA(or tPA) generated by HGF/SF stimulation cleaves plasminogen into the active protease, plasmin. Plasmin in turn cleaves the specific substrate tosyl-glycyl-prolyl-lysine-4-nitranilide acetate (Boehringer Mannheim) or N-methylsulfonyl-D-phenylalanyl-glycyl-arginine-4-nitranilide acetate, generating a chromogenic product which is easily read using an ELISA plate reader (absorbance of 405 nm).

Once compounds are tested for their activity, percent growth relative to control cells was measured (as determined by protein quantitation in replicate plates), such that 100% indicates normal cell growth after a combined assay time of 24 hours, 0% represents the same number of cells as initially plated (i.e. reduced proliferation), and negative values suggest varying degrees of cytotoxicity. Percent inhibition of substrate conversion relative to HGF controls is also quantitated, such that 0% represents no inhibition, and positive values demonstrate inhibition of HGF-induced plasminogen cleavage. Negative values demonstrate enhanced cleavage (i.e. agonistic properties). Percent growth is plotted vs. percent inhibition to identify compounds that display significant inhibition of HGF-mediated plasminogen cleavage without effects on cell proliferation.

Numerous tests using this assay determined the MDCK cells display a high degree of sensitivity and response to human HGF/SF. MDCK cells have the added advantage of scattering in response to HGF/SF stimulation, which allows for visual inspection of Met inhibition to also be determined. As shown in Figure 1, a specific neutralizing antibody against human HGF/SF inhibited HGF/SF-induced plasmin generation in a dose dependent manner. The same antibody did not prevent scattering of the MDCK cells at the same dose of 10 units/ml. Similar results were obtained using anti-human HGF/SF antiserum (clone NCI-53). These results demonstrate the greater sensitivity of the assay over conventional assays and the potential to isolate other compounds that have the capacity to inhibit a HGF/SF phenotype.

We also discovered that geldanamycin (NSC 122750) and an analogue of geldanamycin (NSC 255109) are potent inhibitors in the HGF/SF-Met-urokinase (uPA) cell based screen. Following their initial identification, we performed a number of secondary screens to validate their apparent inhibitory properties. We found that both 122750 and 255109 inhibited HGF/SF-mediated MDCK cell scattering (Fig. 1), HGF/SF-induced SKLMS- I cell motility (Fig. 2), and HGF/SF induced matrigel branching morphogenesis in SKLMS- I cells, U-1 18 human glioma cells and ARZ-2 renal carcinoma cells. Thus, these compounds clearly inhibit cell motility and invasion induced by HGF/SF-Met signaling in a variety of cell types.

We tested the ability of these compounds to revert the transformed phenotype Mediated by Met signaling. These compounds rapidly revert the transformed phenotype of *tpr-met*, HGF/SF-Met and mutationally activated Met transformed cells (Fig. 3). The Met mutations have been identified as familial germline and sporadic mutations in patients with papillary renal carcinoma. Little phenotypic effect was observed upon administration of these compounds to cells transformed by the V12 H-ras oncogene.

We investigated the mechanism(s) by which these compounds inhibit the Met response in these various cell types. We found that both 122750 and 255109 down-regulate the Met receptor (both wild type and mutated), whether endogenously or exogenously expressed (Fig. 4). Thus, the induction of both uPA and the uPA receptor that occurs in both MDCK cells and SKLMS- 1 cells in response to HGF/SF stimulation is blocked by both 122750 and 255109 (Fig. 5), consistent with their activities in the cellular screen. The primary mechanism of action for these drugs is through down-regulation of the Met receptor.

Using the same screening method, we screened 18 geldanamycin analogues in the MDCK cell Met-uPA assay. The majority of these analogues displayed potent inhibition at concentrations as low as 0.03 pM, with little or no cytotoxicity at these low concentrations. In addition, geldanamycin (NSC #122750) has potent activity at these very low concentrations. Some of the analogues am more potent inhibitors of Met-uPA at levels significantly below cytotoxicity levels (e.g., NSC 320877).

In summary, we have identified the geldanamycins as potent inhibitors of the Met protease and invasion pathway at concentrations orders of magnitude below their original and current expectations for clinical applications as cytotoxic/cytostatic drugs. These compounds, at very low concentrations, prevent scattering and invasion in vitro, down-regulate Met expression, and prevent a variety of HGF/SF-mediated responses in different human tumor cell types. These compounds are also potent inhibitors of the transformed phenotype induced by aberrant Met signaling, including cells transformed by mutationally activated Met, that occurs in patients with papillary renal carcinoma. Geldanamycin should therefore be an inhibitor of tumor cell invasion and protease activity (with little or no toxicity) and will be useful in the treatment of certain cancers. Met is known to be an important mediator of metastasis, and since these drugs display anti-invasive characteristics, they can be useful as anti-metastatic agents.

What is claimed is:

1. A method of treating a subject exhibiting a HGF/SF-induced Met-activated metastatic phenotype comprising administering geldanamycin or a geldanamycin derivative to the subject, whereby the geldanamycin or the geldanamycin derivative inhibit Met, thereby treating the subject exhibiting the HGF/SF-induced Met-activated metastatic phenotype.

2. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 255104-U.

3. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 255105-V.

4. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 255110-A.

5. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 255112-F.

6. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 265482-S.

7. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 320877-N.

8. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 330499-V.

9. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 330500-N.

10. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 330507-C.

11. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 330512-L.

12. The method of claim 1, wherein the geldanamycin derivative comprises NSC # D658514-K.

13. The method of claim 1, wherein the geldanamycin derivative comprises NSC # D661581-S.

14. The method of claim 1, wherein the geldanamycin derivative comprises NSC # D662199-F.

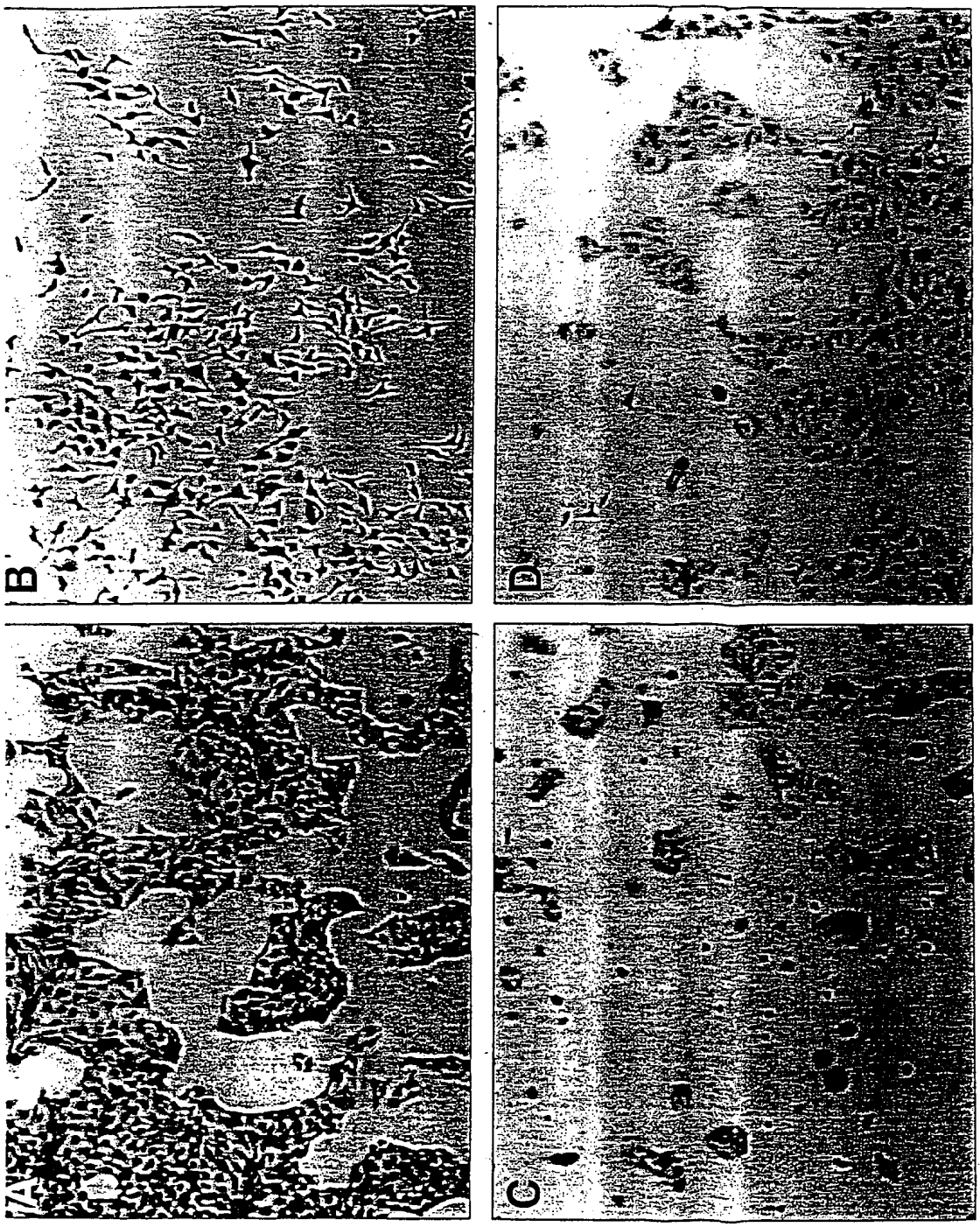
15. The method of claim 1, wherein the geldanamycin derivative comprises NSC # D674124-A.

16. The method of claim 1, wherein the geldanamycin derivative comprises NSC # D683201-S.

17. The method of claim 1, wherein the geldanamycin derivative comprises NSC # D697886-G.

18. The method of claim 1, wherein the geldanamycin derivative comprises NSC # 255109E.

19. The use of geldanamycin, The method of claim 1, wherein the geldanamycin derivative comprises NSC # 255104-U, NSC # 255105-V, NSC # 255110-A, NSC # 255112-F, NSC # 265482-S, NSC # 320877-N, NSC # 330499-V, NSC # 330500-N, NSC # 330507-C, NSC # 330512-L, NSC # D658514-K, NSC # D661581-S, NSC # D662199-F, NSC # D674124-A, NSC # D683201-S, NSC # D697886-G, or NSC # 255109E. for the manufacture of a medicament for inhibiting a hepatocyte growth factor/scatter factor (HGF/SF) induced phenotype in a cell.



A=Control MDCK Cells
B=MDCK Cells + 24 hr HGF/SF
C=MDCK Cells + 24 hr HGF/SF + 10 nM 122750
D=MDCK Cells + 24 hr HGF/SF + 10 nM 255109

FIG. 1

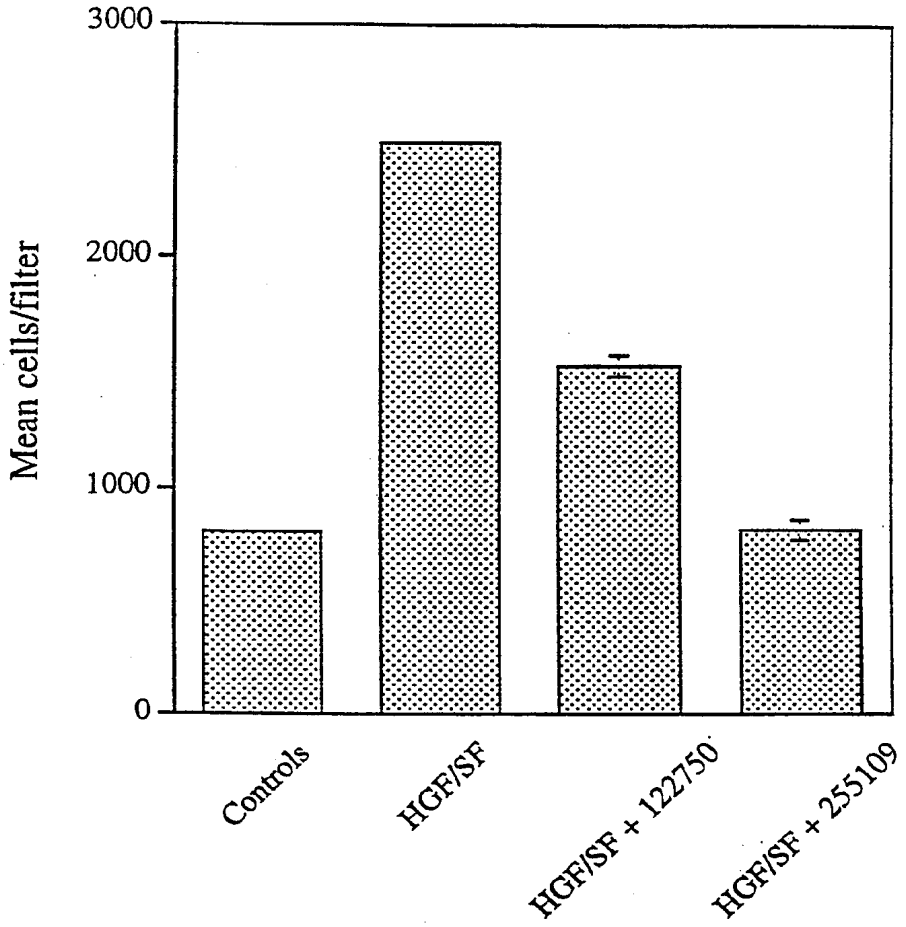

3000
2000
1000
0
Mean cells/filter
Controls
HGF/SF
HGF/SF + 122750
HGF/SF + 255109

FIG. 2

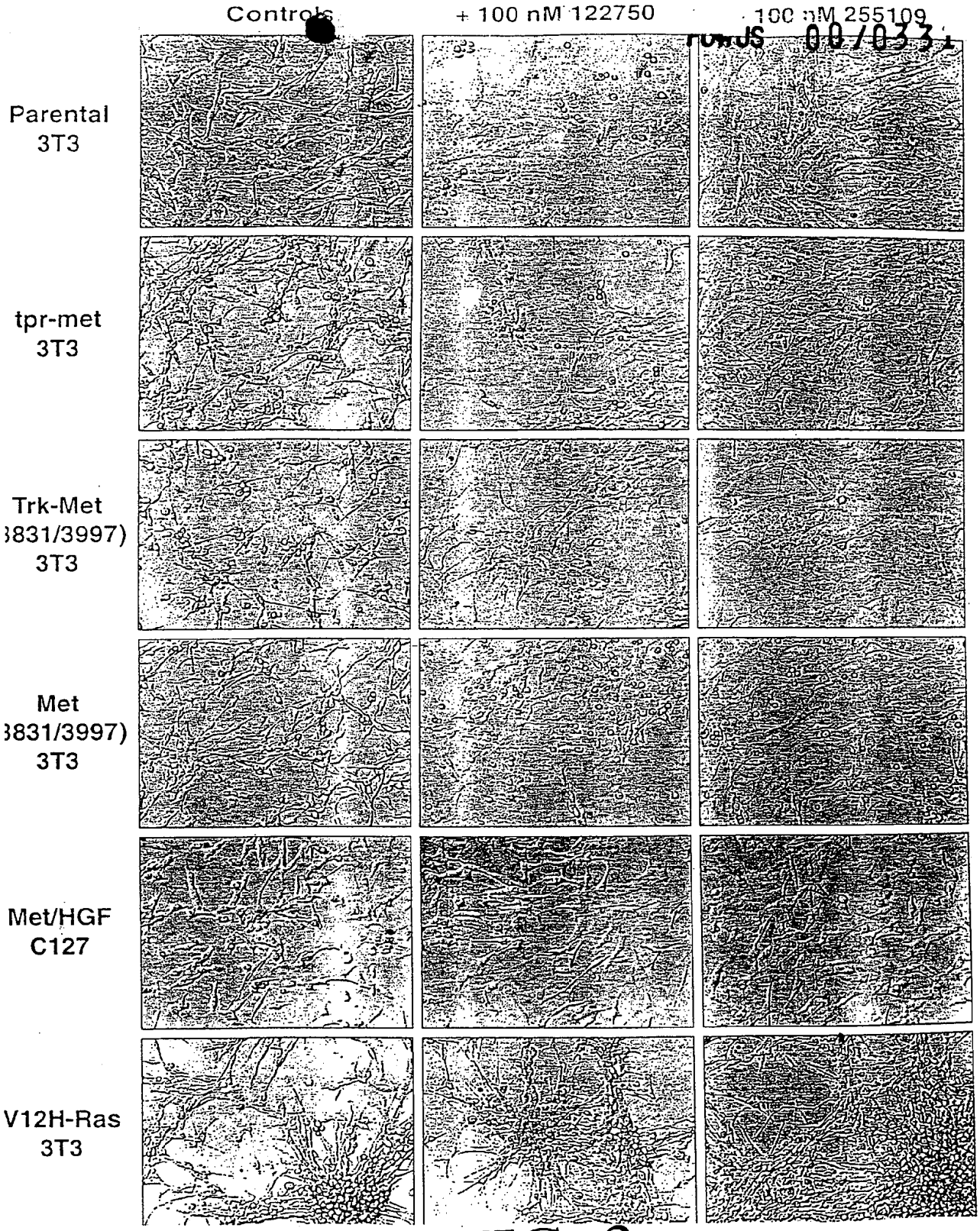

Controls
+ 100 nM 122750
100 nM 255109
Parental 3T3
tpr-met 3T3
Trk-Met 3831/3997) 3T3
Met 3831/3997) 3T3
Met/HGF C127
V12H-Ras 3T3

FIG. 3

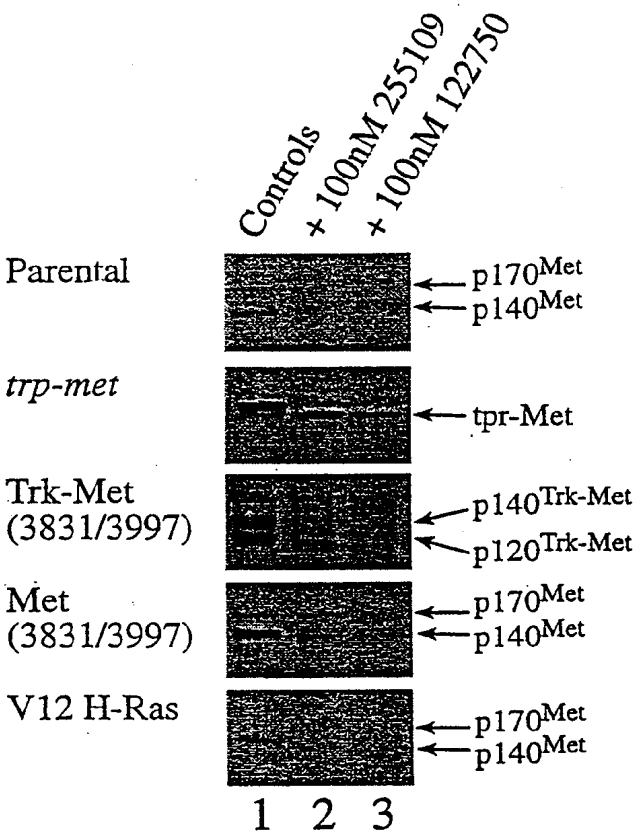

Controls
+ 100nM 255109
+ 100nM 122750
Parental
p170Met
p140Met
trp-met
tpr-Met
Trk-Met
(3831/3997)
p140Trk-Met
p120Trk-Met
Met
(3831/3997)
p170Met
p140Met
V12 H-Ras
p170Met
p140Met
1 2 3

FIG. 4

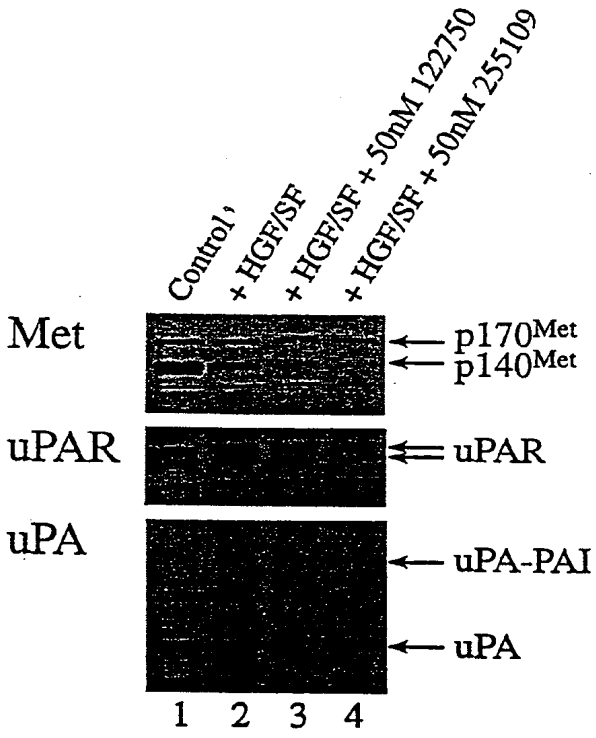
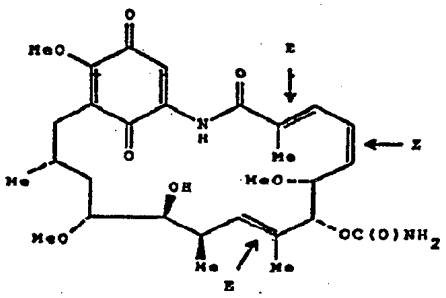

Control'
+ HGF/SF
+ HGF/SF + 50nM 122750
+ HGF/SF + 50nM 255109
Met
p170Met
p140Met
uPAR
uPAR
uPA
uPA-PAI
uPA
1 2 3 4

FIG. 5

NSC Number 122750-I
(CNAM) Chemical Name: Antibiotic (U-29,135);
Antibiotic U 29135;
Geldanamicin;
Geldanamycin;
GELDANAMYCIN



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	8.7	10.4	81.1	14.4
3.00E-13	7.8	14.1	76.8	18.4
3.00E-12	5.3	12.2	65.9	15.1
3.00E-11	8.1	9.2	82.2	7.2
3.00E-10	12.1	10.3	93.6	24.2
3.00E-09	15.3	6.8	105.4	15.7
3.00E-08	16.7	10.5	107.6	14.2
3.00E-07	21.9	8.7	108.7	7.2
3.00E-06	23.4	10.4	109.6	5.6
3.00E-05	86.7	5.4		

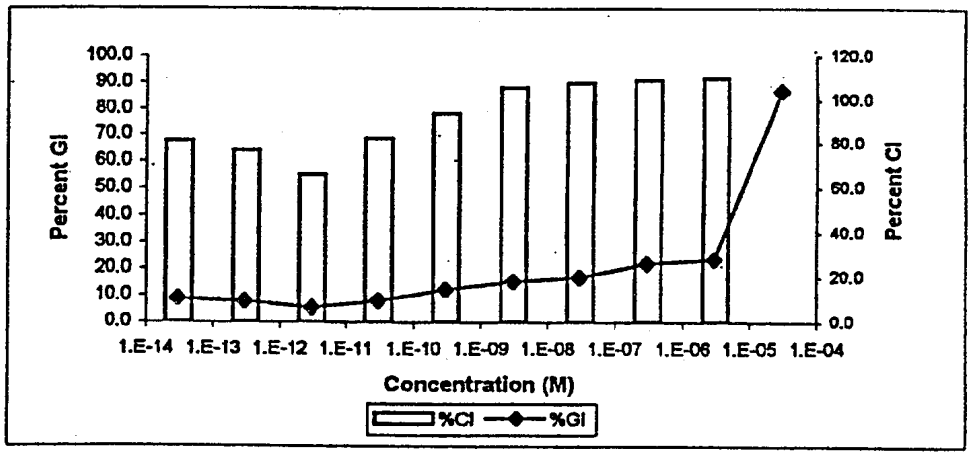
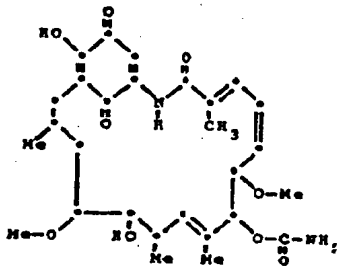


FIG. 6

NSC Number 255104-U
(CNAM) Chemical Name: Des-O-methylgeldanamycin;
GELDANAMYCIN ANALOG;
17-Des-O-methylgeldanamycin;
17-Desmethylgeldanamycin



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	0.9	12.9	18.5	19.6
3.00E-13	-1.2	7.2	0.8	7.4
3.00E-12	-5.1	10.7	-4.5	4.2
3.00E-11	-2.3	13.0	-3.8	24.5
3.00E-10	-3.7	11.5	2.9	32.2
3.00E-09	6.2	6.1	64.6	37.5
3.00E-08	3.8	5.6	80.6	31.1
3.00E-07	18.0	2.5	97.3	9.0
3.00E-06	20.2	0.9	102.9	9.8
3.00E-05	27.0	4.4	115.6	4.9

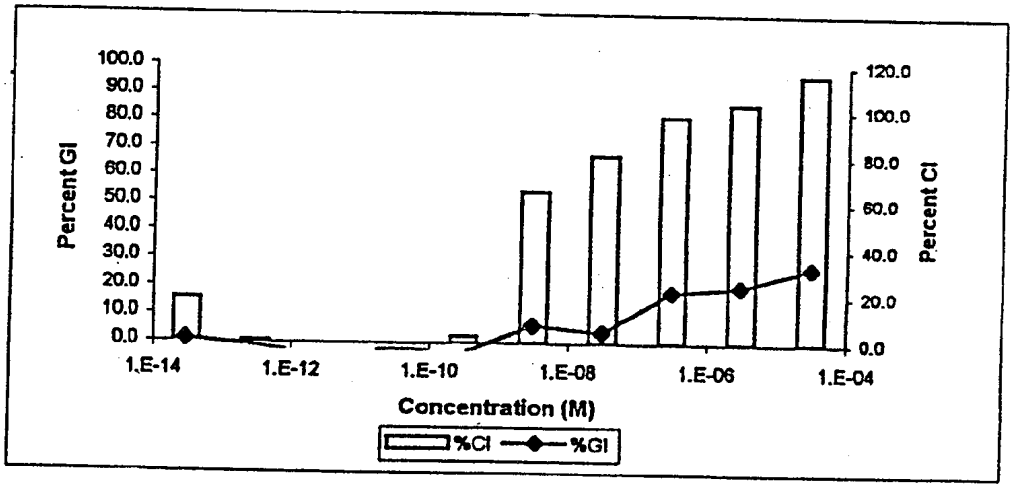
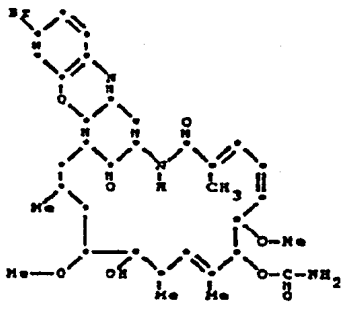


FIG. 7

NSC Number 255105-V
(CNAM) Chemical Name: GELDANAMYCIN ANALOG;
7'-Bromodemethoxy geldanoxazone;
7'-Bromodemethoxygeldanoxazone



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	2.7	10.5	86.5	16.3
3.00E-13	4.8	9.6	88.7	1.1
3.00E-12	2.2	9.4	64.5	43.1
3.00E-11	2.7	16.9	65.6	30.7
3.00E-10	1.7	10.3	64.9	33.8
3.00E-09	10.4	6.1	94.6	15.0
3.00E-08	14.1	4.7	102.9	10.9
3.00E-07	18.8	8.5	104.6	10.2
3.00E-06	21.7	6.4	108.9	9.5
3.00E-05	81.2	2.8		

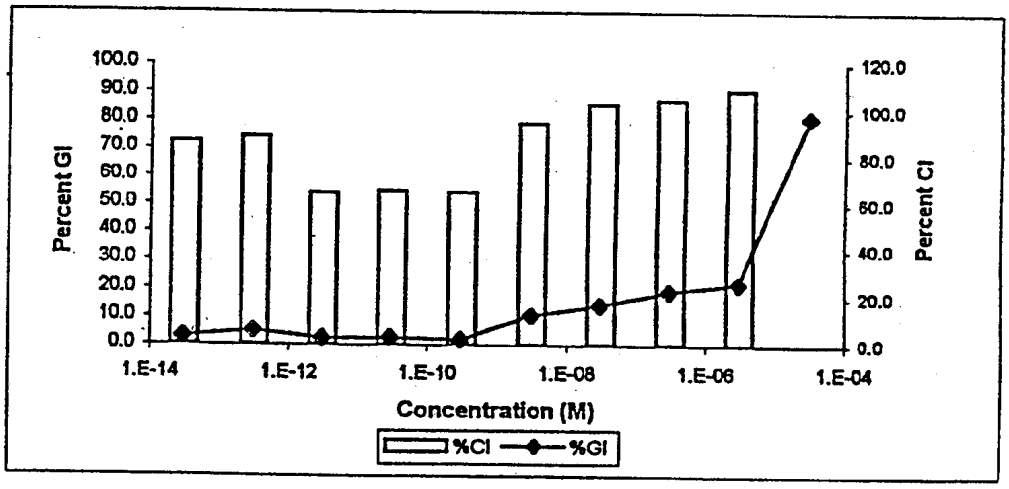
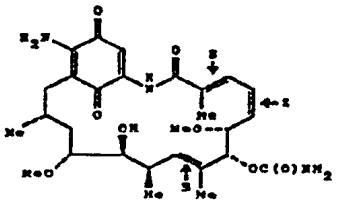


FIG. 8

NSC Number 255109-2
(CHAN) Chemical Name: Geldanamycin, 17-amino-17-demethoxy-;
GELDANAMYCIN ANALOG;
17-Amino-17-demethoxygeldanamycin;
17-Aminodemethoxygeldanamycin



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	5.9	4.4	81.0	9.8
3.00E-13	6.9	10.5	79.0	20.0
3.00E-12	7.1	7.6	79.5	26.0
3.00E-11	5.4	16.4	66.8	41.6
3.00E-10	11.3	10.1	93.3	23.3
3.00E-09	20.1	7.0	106.6	13.6
3.00E-08	20.7	7.5	109.0	11.3
3.00E-07	22.3	8.5	111.1	9.9
3.00E-06	25.5	5.5	104.8	10.6
3.00E-05	50.9	6.5		

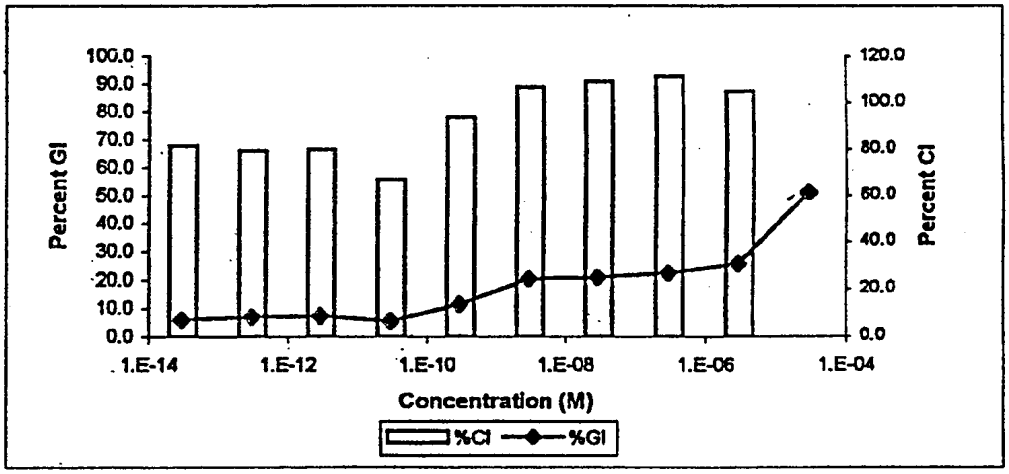
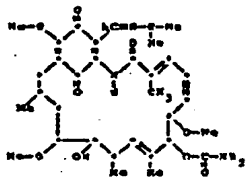


FIG. 9

NSC Number 253110-A
(CHAN) Chemical Name: 19-Formylgeldanamycin N',N'-dimethyl-hydrazone; 19-Formylgeldanamycin-N',N'-dimethylhydrazone



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-8.7	1.2	1.1	7.1
3.00E-13	-8.4	5.3	6.6	8.1
3.00E-12	-6.0	0.3	-10.3	20.4
3.00E-11	-11.7	8.5	-1.4	10.0
3.00E-10	-6.9	1.1	-7.5	20.8
3.00E-09	-2.1	4.3	12.4	13.4
3.00E-08	-1.7	9.4	12.1	14.3
3.00E-07	0.6	12.9	3.3	23.3
3.00E-06	4.2	7.4	2.8	22.7
3.00E-05	25.6	4.7	127.5	

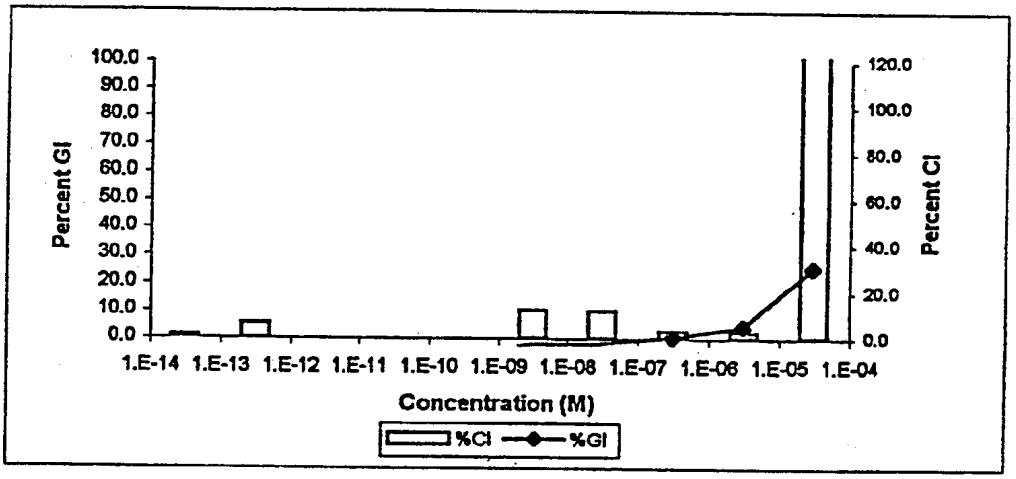
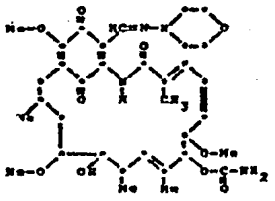


FIG. 10

NSC Number 755112-F
(CHAM) Chemical Name: 19-Formylgeldanamycin N-morpholinoimine; 19-Formylgeldanamycin-N-aminomorpholine adduct



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-6.3	1.0	3.0	14.6
3.00E-13	-5.1	1.5	3.4	4.2
3.00E-12	-5.2	1.5	-6.1	19.3
3.00E-11	-8.3	4.1	-4.6	14.3
3.00E-10	-7.4	1.7	-6.5	18.0
3.00E-09	-2.7	7.4	11.6	17.4
3.00E-08	-6.8	11.9	14.4	20.3
3.00E-07	-5.0	8.5	9.2	16.7
3.00E-06	-2.7	8.4	15.0	22.7
3.00E-05	16.5	1.6	111.4	10.9

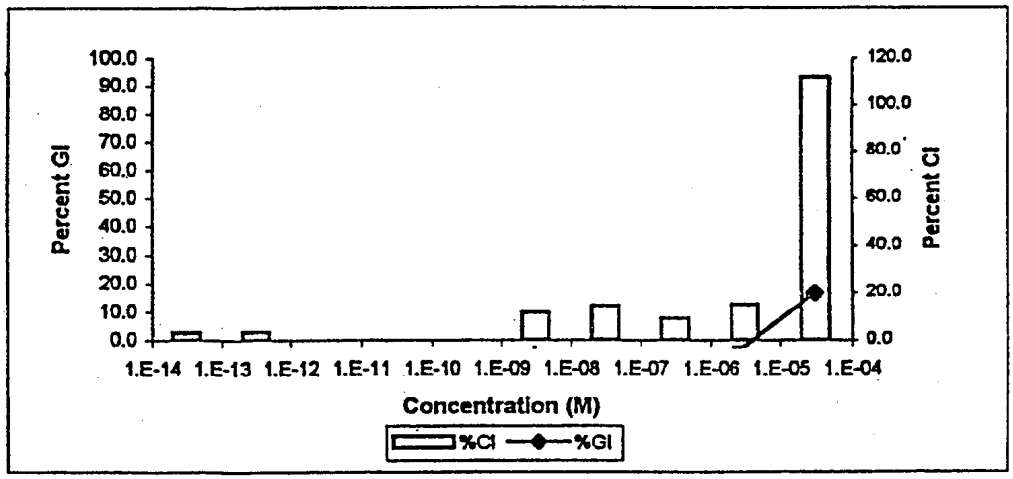
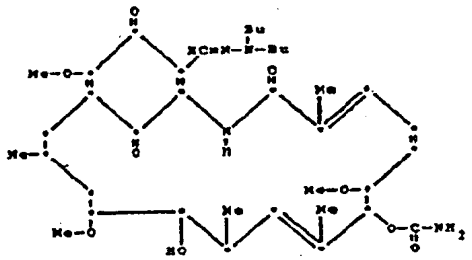


FIG. 11

NSC Number 263482-S
(CHAM) Chemical Name: VL GELD 33;
19-Formylgeldanamycin N',N'-dibutylhydrazone



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-6.6	2.8	1.9	3.5
3.00E-13	-3.9	2.6	6.6	5.9
3.00E-12	-2.7	3.5	-5.5	11.0
3.00E-11	-2.9	2.3	-6.5	9.1
3.00E-10	-5.4	3.6	-7.1	18.3
3.00E-09	-2.4	2.6	14.7	10.8
3.00E-08	1.6	6.1	8.7	7.2
3.00E-07	1.0	13.9	6.0	21.5
3.00E-06	7.1	6.2	52.8	21.7
3.00E-05	54.1	1.3		

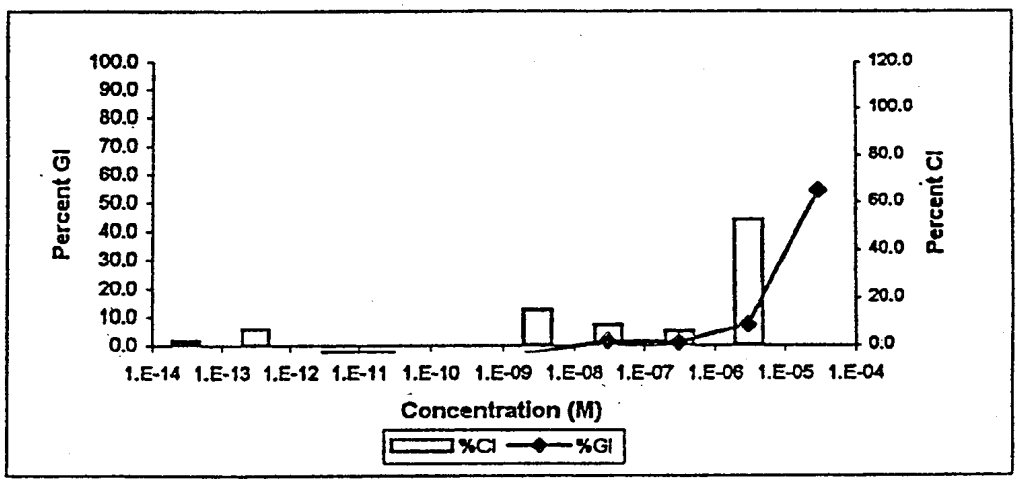
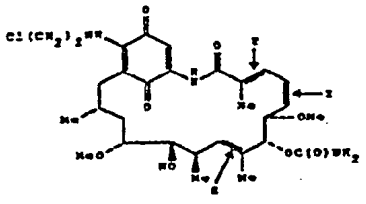


FIG. 12

NSC Number 320877-M
(CAS) Chemical Name: Geldanamycin, 17-[(2-chloroethyl)amino]-17-demethoxy-;
GELDANAMYCIN DERIV



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	0.7	5.2	97.0	3.8
3.00E-13	2.4	6.2	87.7	1.6
3.00E-12	4.6	0.5	76.8	3.4
3.00E-11	1.1	9.0	76.2	5.7
3.00E-10	1.9	5.2	73.1	6.6
3.00E-09	15.6	5.8	99.6	7.1
3.00E-08	13.8	6.4	106.9	7.6
3.00E-07	17.1	7.2	111.0	10.5
3.00E-06	41.8	10.8		
3.00E-05	68.1	9.3		

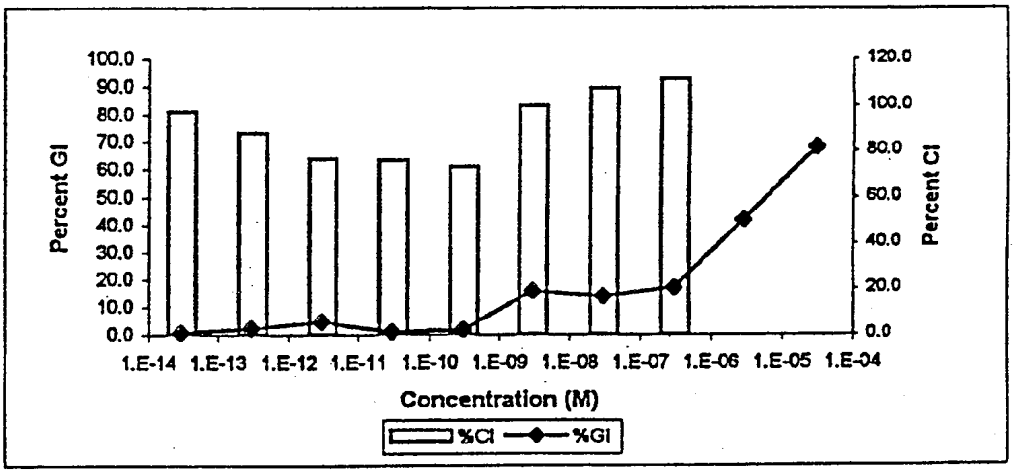
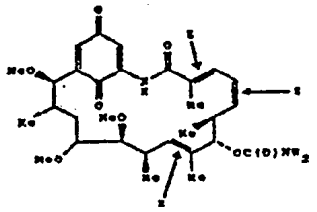


FIG. 13

NSC Number 330499-V
(CHEM) Chemical Name: Geldanamycin, 6,17-didemethoxy-19-methoxy-6-methyl-11-O-methyl-, (6S,19R)- (9CI);
Macbecin I;
MACBECIN I;
2-Azabicyclo[16.3.1]docosane, geldanamycin deriv. (9CI)



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-1.3	1.6	14.3	25.4
3.00E-13	-2.2	2.9	1.7	37.8
3.00E-12	1.7	3.4	-10.4	56.5
3.00E-11	2.3	6.1	-15.7	53.6
3.00E-10	2.0	16.5	-20.7	39.7
3.00E-09	-3.0	1.9	18.6	8.1
3.00E-08	-3.7	1.9	21.2	11.3
3.00E-07	9.4	1.0	65.3	9.9
3.00E-06	26.2	8.4	105.2	19.2
3.00E-05	82.7	3.7		

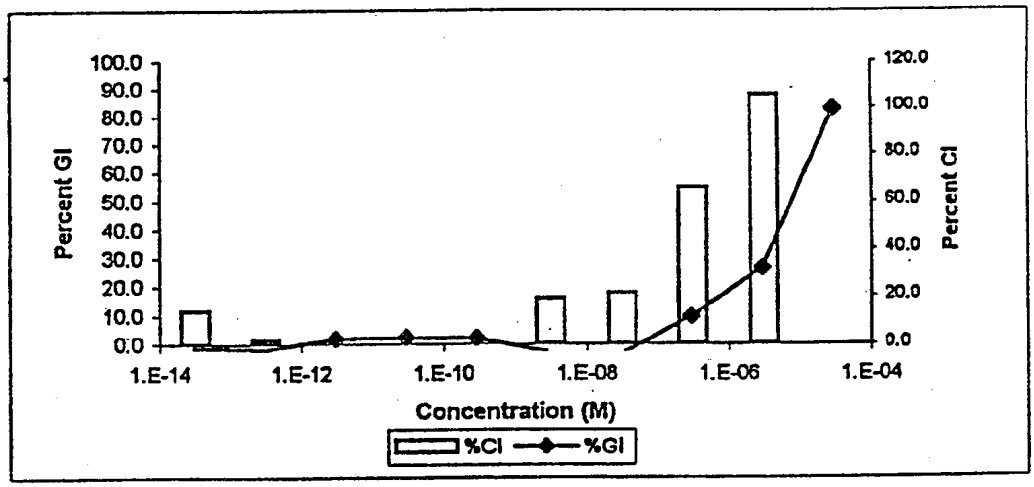
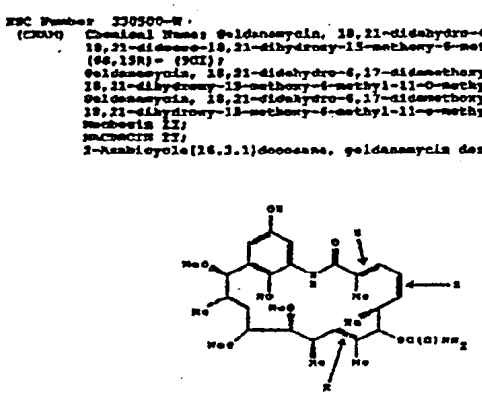


FIG. 14



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-2.6	1.5	5.9	22.1
3.00E-13	0.5	3.7	-10.5	32.7
3.00E-12	2.6	3.5	-10.6	57.1
3.00E-11	-2.7	11.0	-10.3	54.0
3.00E-10	6.5	12.5	-35.0	46.7
3.00E-09	-4.2	8.0	12.6	17.4
3.00E-08	-3.6	7.3	16.8	18.7
3.00E-07	3.9	9.7	77.6	8.4
3.00E-06	16.3	2.0	100.9	10.4
3.00E-05	81.7	3.5		

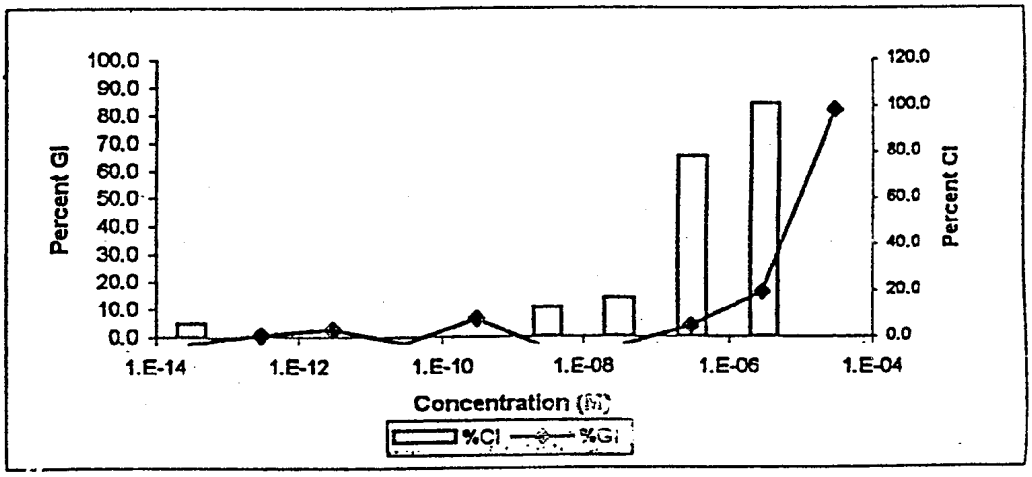
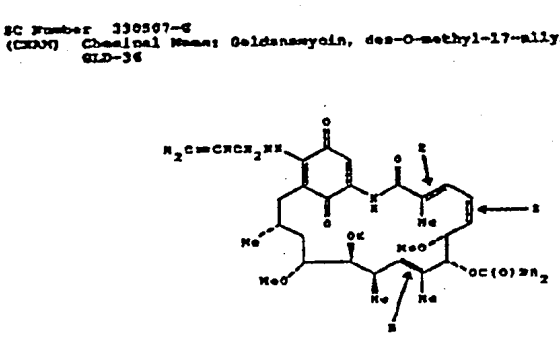


FIG. 15



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	7.8	3.9	99.4	1.8
3.00E-13	8.7	4.0	87.9	11.8
3.00E-12	7.4	6.7	74.5	28.6
3.00E-11	8.3	9.9	75.0	23.1
3.00E-10	12.1	15.5	75.0	24.5
3.00E-09	7.5	4.3	100.6	9.0
3.00E-08	7.8	2.9	103.0	9.1
3.00E-07	13.0	2.5	104.7	6.4
3.00E-06	15.2	1.6	103.5	6.7
3.00E-05	49.6	4.5		

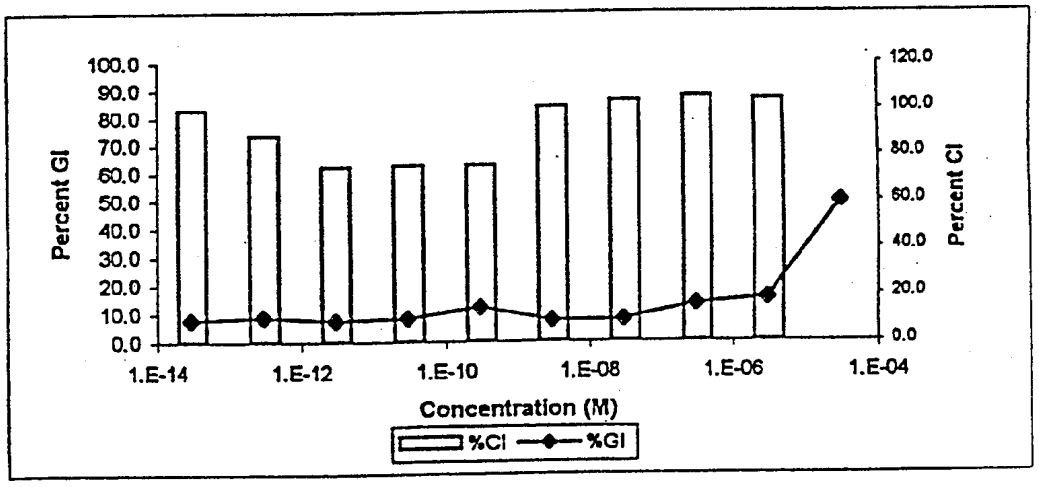
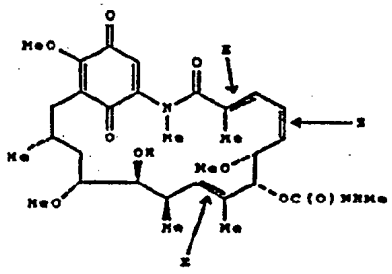


FIG. 16

SC Number 330512-L
(CNAM) Chemical Name: GELDANAMYCIN, N,N'-DIMETHYL; GLD-91-III



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-5.7	16.9	0.5	25.2
3.00E-13	4.6	4.9	-3.3	16.1
3.00E-12	9.5	7.4	-23.2	29.6
3.00E-11	-0.8	1.6	-2.3	12.9
3.00E-10	4.9	4.7	-13.1	17.4
3.00E-09	1.4	7.0	39.7	58.8
3.00E-08	-1.3	9.8	38.0	65.0
3.00E-07	3.0	4.9	76.2	44.2
3.00E-06	6.6	7.5	102.6	15.7
3.00E-05	40.8	18.9	118.8	

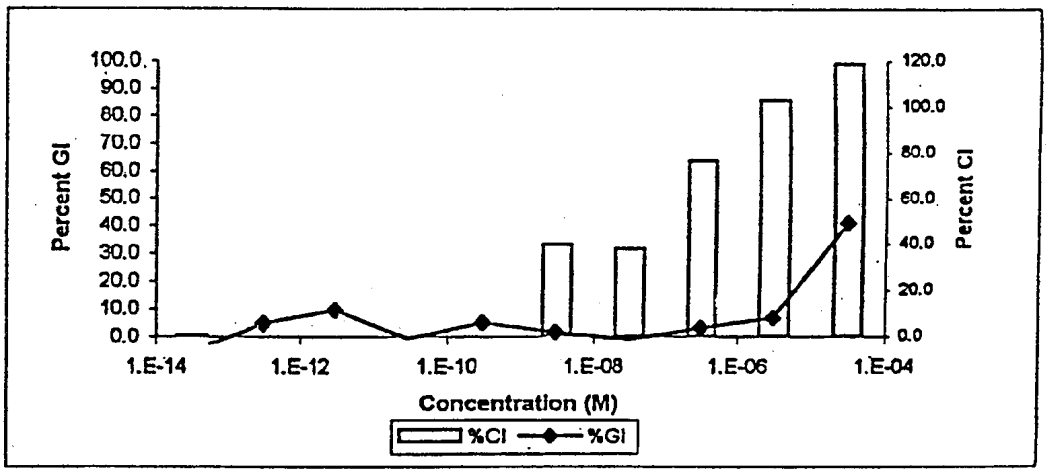
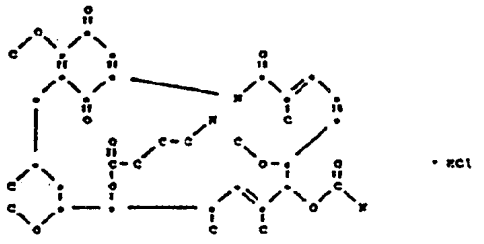


FIG. 17

NSC Number D658514-K
(CAS#) Chemical Name: Geldanamycin 4-aminobutyrate hydrochloride



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-2.5	15.1	-5.7	23.5
3.00E-13	5.5	6.8	-9.0	8.2
3.00E-12	1.9	4.3	-18.6	21.4
3.00E-11	4.5	1.0	-6.1	3.7
3.00E-10	2.5	0.1	-16.4	17.0
3.00E-09	4.8	18.0	42.4	63.9
3.00E-08	6.0	22.3	32.3	72.3
3.00E-07	2.3	3.5	64.2	50.5
3.00E-06	7.2	6.4	101.0	20.4
3.00E-05	72.0	18.7		

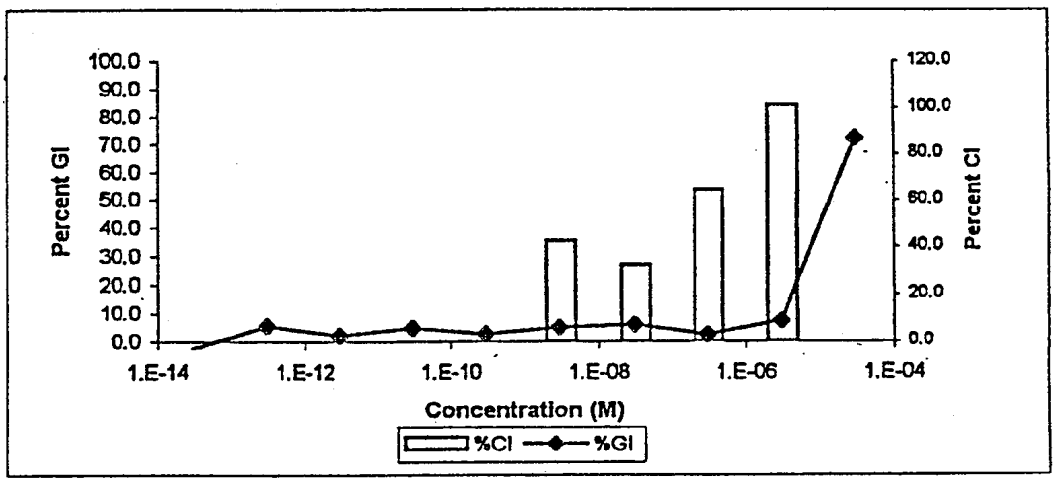
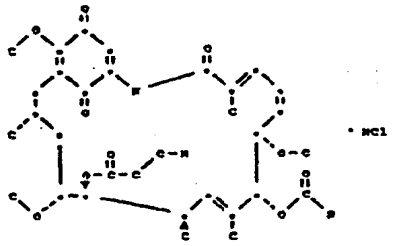


FIG. 18

NSC Number D661581-2
(CHAM) Chemical Name: Geldanamycin, 12-(3-amisopropionate)-monohydrochloride; 2-Azabicyclo[16.3.1]docosa-4,6,10,18,21-pentaene- 3,20,23-trione, 13-(3-amisopropionyloxy)-9,13-dihydroxy- 8,14,19-trimethoxy-4,10,12,16-tetramethyl-, 9-carbamate, monohydrochloride; 2-Azabicyclo[16.3.1]docosane, geldanamycin deriv.;



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-0.2	3.6	3.0	26.1
3.00E-13	9.3	2.8	4.3	28.8
3.00E-12	5.1	1.6	-18.4	19.4
3.00E-11	5.3	0.8	5.3	19.7
3.00E-10	1.3	12.4	-24.8	30.1
3.00E-09	8.2	18.4	36.6	64.7
3.00E-08	6.5	24.5	49.9	63.7
3.00E-07	5.9	4.2	66.7	62.2
3.00E-06	20.9	8.8	106.7	12.3
3.00E-05	81.7	4.1		

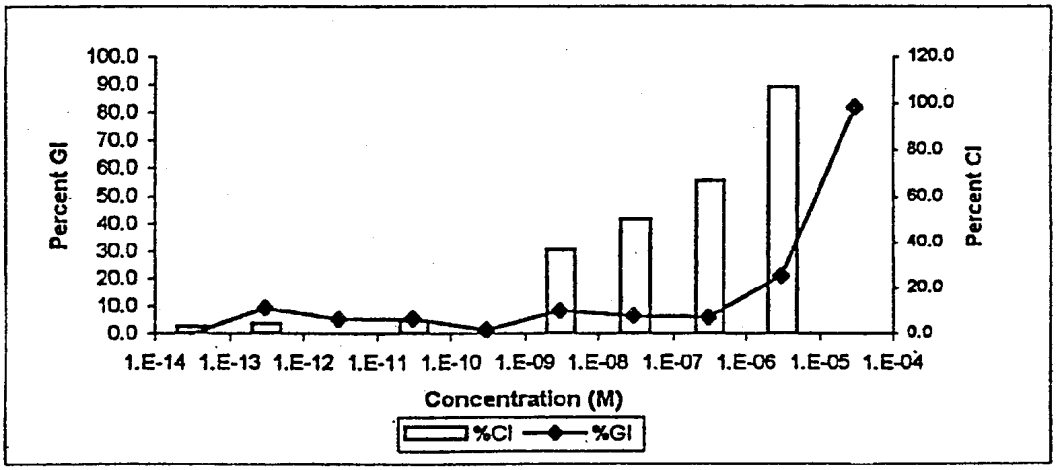
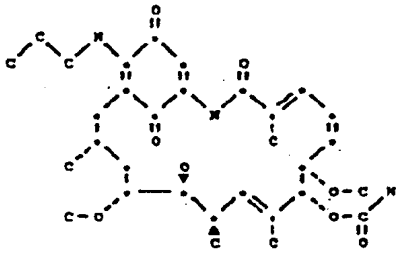


FIG. 19

NSC Number D662199-P
(CHAM) Chemical Name: 17-n-Propylamino-geldanamycin



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-2.0	10.0	62.3	18.9
3.00E-13	0.0	11.8	26.4	23.4
3.00E-12	2.1	9.5	25.5	19.4
3.00E-11	3.2	7.7	36.2	10.6
3.00E-10	3.9	18.2	28.0	28.8
3.00E-09	2.4	8.7	96.5	17.2
3.00E-08	5.7	8.2	101.4	17.7
3.00E-07	10.5	6.6	108.4	9.1
3.00E-06	15.8	11.0	97.9	20.8
3.00E-05	42.7	11.4		

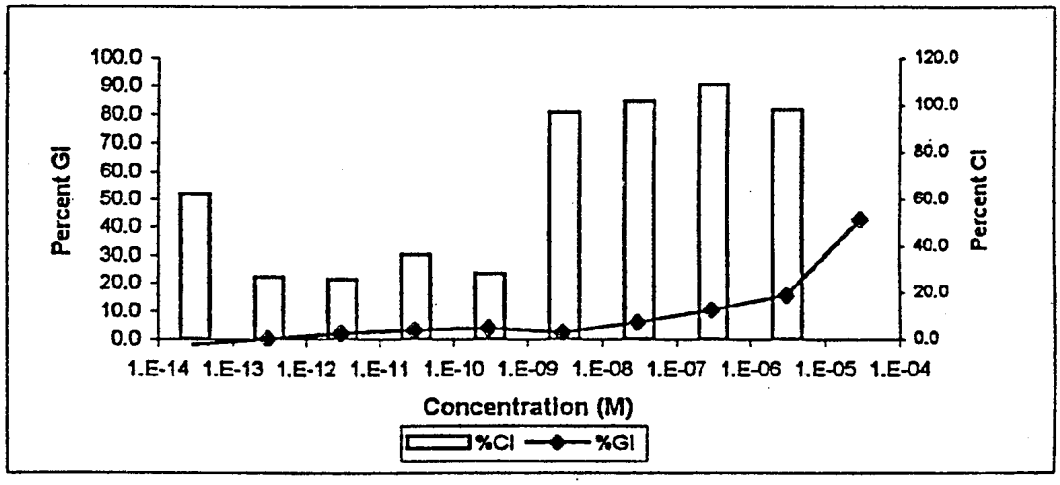
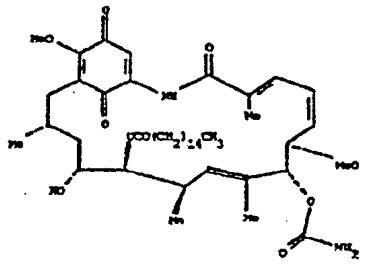


FIG. 20

NSC Number D674124-A
(CHAM) Chemical Name: Geldanamycin-11-hexadecanic acid ester



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-1.7	3.9	3.4	2.5
3.00E-13	2.7	9.7	-3.7	5.6
3.00E-12	-3.2	0.1	-2.2	1.1
3.00E-11	-2.6	6.7	-2.7	6.2
3.00E-10	2.1	2.3	-17.5	13.8
3.00E-09	0.8	1.8	-0.2	2.4
3.00E-08	2.1	2.0	-6.5	3.8
3.00E-07	5.4	10.5	-13.0	12.2
3.00E-06	6.9	15.4	35.4	73.2
3.00E-05	2.5	6.9	39.9	44.2

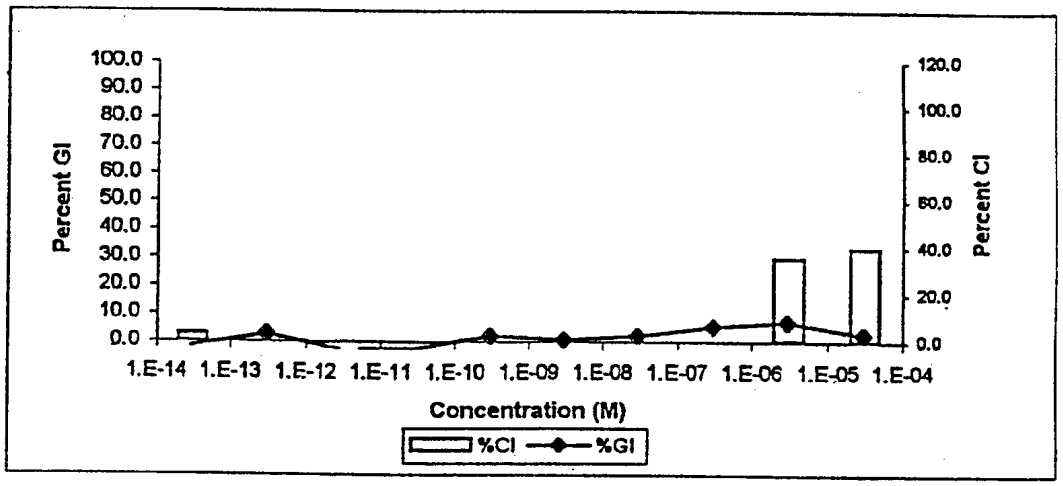
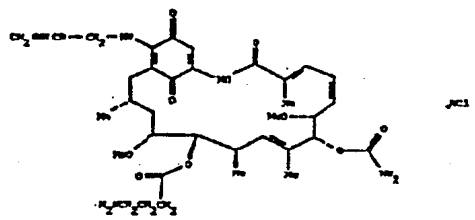


FIG. 21

NSC Number D683201-6
(CHAN) Chemical Name: 17-Demethoxy-17-N-allylamino-geldanamycin 11-(4'-aminobutyrate) hydrochloride;



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-3.8	1.5	0.0	2.3
3.00E-13	1.3	8.2	-5.3	10.4
3.00E-12	-4.6	2.8	1.4	2.4
3.00E-11	-3.5	3.7	-9.4	10.9
3.00E-10	-1.1	2.6	-14.3	8.8
3.00E-09	1.5	2.9	3.4	7.5
3.00E-08	0.5	6.6	-0.2	2.7
3.00E-07	4.9	7.2	-1.3	14.6
3.00E-06	4.6	4.8	48.9	58.1
3.00E-05	59.5	17.8		

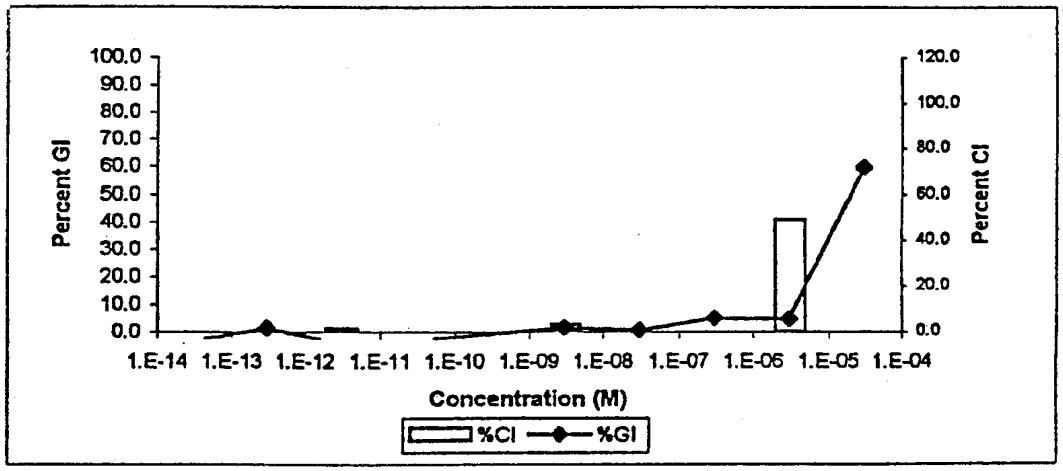
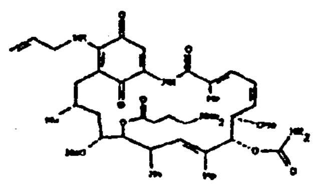


FIG. 22

NSC Number D697886-G
(CIDUQ Chemical Name: 17-Demethoxy-17-allylamino geldanamycin-11-[4-(dimethylamino)butyrate];



Concentration (Molar)	%GI	STD %GI	%CI	STD %CI
3.00E-14	-7.7	6.9	6.8	12.8
3.00E-13	-0.3	11.6	-2.7	14.0
3.00E-12	-10.4	6.8	6.8	5.2
3.00E-11	-4.3	6.1	-6.0	15.8
3.00E-10	-1.2	4.1	-13.5	9.9
3.00E-09	-4.4	2.0	21.4	30.6
3.00E-08	5.5	16.1	30.4	54.7
3.00E-07	2.6	14.3	38.5	69.1
3.00E-06	5.9	6.1	93.7	19.5
3.00E-05	61.6	11.4		

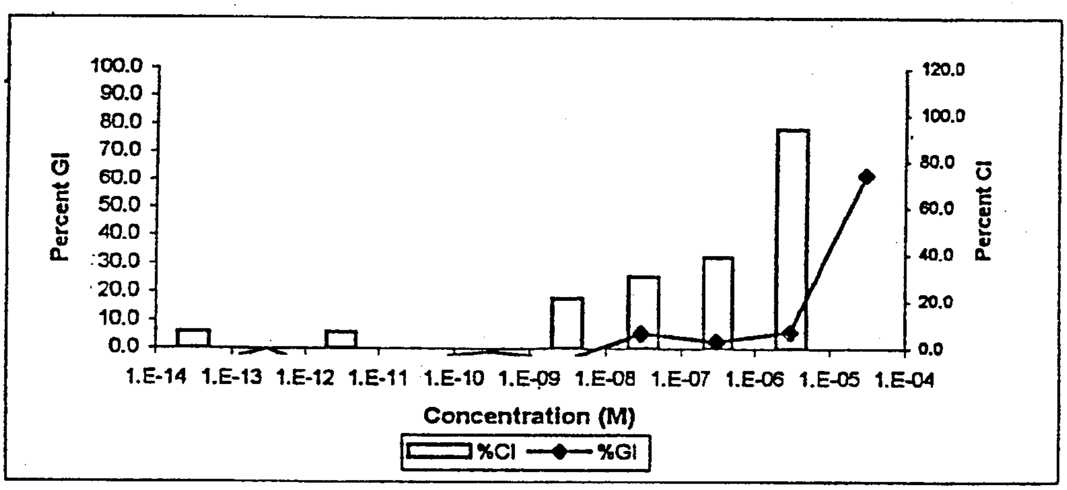


FIG. 23